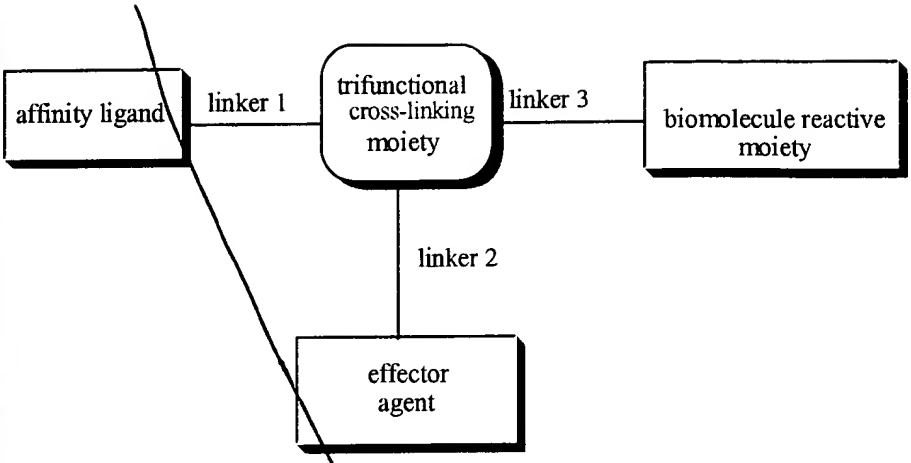


the single molecule reagent comprising:

a trifunctional cross-linking moiety, which is optionally tetrafunctional;

an affinity ligand, coupled to the trifunctional cross-linking moiety via a first linker which is optionally stabilized to inhibit enzymatic cleavage of the affinity ligand,

the affinity ligand having an affinity to bind specifically to at least one member selected from the group consisting of avidin; a derivative, mutant or fragment of avidin having essentially the same binding function to the affinity ligand as avidin; streptavidin; and a derivative, mutant or fragment of streptavidin having essentially the same binding function to the affinity ligand as streptavidin, the chosen member exhibiting an affinity constant of at least 10^6 M^{-1} toward the affinity ligand;

an effector agent, coupled to the tri-functional cross-linking moiety by a covalent bond or by a second linker, the effector agent having an in vivo, ex vivo or in

vitro effect on at least one member selected from the group consisting of a cell, a tissue, and a humoral molecule other than a biomolecule linked to the tri-functional cross-linking moiety, wherein the effector agent is selected from the group consisting of a synthetic or naturally occurring toxin; an enzyme, optionally capable of converting a pro-drug to an active drug; a hormone; an immunosuppressive agent; an immunostimulating agent; a radionuclide binding/bonding moiety to which is optionally bound or chelated a radiosensitizer, an enhancer for an X-ray, MRI or ultrasound technique, or a non-radioactive element which can be converted to a radioactive element by means of external irradiation; a photoactive compound; a compound used in photoimaging; and a compound used in photodynamic therapy; and

a biomolecule reactive moiety, coupled to the trifunctional cross-linking moiety, optionally via a third linker, the biomolecule reactive moiety being able to react with a biomolecule to form a covalent bond with the biomolecule.

34. The single molecule reagent according to claim 33, wherein the trifunctional cross-linking moiety comprises a member selected from the group consisting of triaminobenzene, tricarboxybenzene, dicarboxyaniline and diaminobenzoic acid.

35. The single molecule reagent according to claim 33, wherein the affinity ligand comprises biotin or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin.

36. The single molecule reagent according to claim 33, wherein the affinity ligand comprises

a biotin derivative selected from the group consisting of norbiotin, homobiotin, oxybiotin, imino-biotin, desthiobiotin, diaminobiotin, biotin sulfoxide, and biotin sulfone.

37. The single molecule reagent according to claim 33, wherein the affinity ligand comprises a biotin derivative which inhibits bioinidase from enzymatically cleaving the biotinamide bond.

38. The single molecule reagent according to claim 37, wherein the biotin derivative is selected from the group consisting of norbiotin and homobiotin.

39. The single molecule reagent according to claim 35, wherein the first linker serves as a spacer between the trifunctional cross-linking moiety and the biotin moiety such that binding to the biotin moiety is not diminished by steric hindrance.

40. The single molecule reagent according to claim 35, wherein the first linker comprises at least one member selected from the group consisting of compounds containing hydrogen-bonding atoms, and compounds containing ionizable groups, to thereby increase the water solubility of the biotin moiety.

41. The single molecule reagent according to claim 40, wherein the compound containing hydrogen-bonding atoms comprises a member selected from the group consisting of ethers and thioethers.

42. The single molecule reagent according to claim 40, wherein the ionizable groups are

selected from the group consisting of carboxylates, sulfonates, and ammonium groups.

43. The single molecule reagent according to claim 35, wherein the first linker comprises an alpha carboxylate, or an N-methyl group, to thereby stabilize a biotinamide bond against enzymatic cleavage by biotinidase.

44. The single molecule reagent according to claim 33, wherein the effector agent comprises an amino-carboxy derivative or a cyclic amine.

45. The single molecule reagent according to claim 44, wherein the amino-carboxy derivative is selected from the group consisting of an EDTA derivative and a DTPA derivatives.

B1

46. The single molecule reagent according to claim 44, wherein the amino-carboxy derivative is selected from the group consisting of Me-DTPA, CITC-DTPA, and cyclohexyl-DTPA.

47. The single molecule reagent according to claim 44, wherein the cyclic amine is selected from the group consisting of NOTA, DOTA, and TETA, and wherein the effector agent comprises a member selected from the group consisting of In, Y, Pb, Bi, Cu, Sm, and Lu radionuclides.

48. The single molecule reagent according to claim 33, wherein the effector agent comprises a member selected from the group consisting of a positron imaging radionuclide, a therapeutic radionuclide, and a gamma imaging radionuclide.

49. The single molecule reagent according to claim 48, wherein the positron imaging radionuclide comprises a member selected from the group consisting of F-18, Br-75, Br-76, and I-124.

50. The single molecule reagent according to claim 48, wherein the therapeutic radionuclide comprises a member selected from the group consisting of Y-90, I-131, In-114m, Re-186, Re-188, Cu-67, Sm-157, Lu-177, Bi-212, Bi-213, At-211, and Ra-223.

51. The single molecule reagent according to claim 48, wherein the gamma imaging radionuclide is selected from the group consisting of Tc-99m, In-111 and I-123.

B1

52. The single molecule reagent according to claim 33, wherein the effector agent comprises a compound which can be converted to a photoactive compound.

53. The single molecule reagent according to claim 52, wherein the compound which can be converted to a photoactive compound comprises a member selected from the group consisting of a chromophore compound and a fluorophore compound.

54. The single molecule reagent according to claim 33, wherein the effector agent is coupled to the tri-functional cross-linking moiety without a second linker.

Subb D1

55. The single molecule reagent according to claim 33, wherein the effector agent is coupled to the tri-functional cross-linking moiety by the second linker which comprises a spacer having a length of 1-25 atoms or groups of atoms.

56. The single molecule reagent according to claim 33, wherein the effector agent is coupled to the tri-functional cross-linking moiety by the second linker which comprises a spacer having a length of 6-18 atoms, or groups of atoms.

57. The single molecule reagent according to claim 33, wherein the second linker aids in water solubility.

58. The single molecule reagent according to claim 57, wherein the second linker comprises a hydrogen-bonding atom.

B1

59. The single molecule reagent according to claim 58 wherein the second linker comprises ethers or thioethers.

60. The single molecule reagent according to claim 57, wherein the second linker comprises an ionizable group.

61. The single molecule reagent according to claim 60, wherein the ionizable group is selected from the group consisting of carboxylates, sulfonates, and ammonium groups.

62. The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety comprises a member selected from the group consisting of an active ester; an N-hydroxy-succinimide ester; a sulfo-N-hydroxysuccinimide ester; a phenolic ester; an aryl imidate; an alkyl imidate; an alkyl isocyanate, an aryl isocyanate or an isothiocyanate which reacts with one or

more amino groups on the biomolecule; a maleimide, or an alpha-haloamide which reacts with one or more sulfhydryl groups on the biomolecule; and an arylhydrazine, an alkylhydrazine, an alkyl hydroxylamine or an aryl hydroxylamine which reacts with one or more aldehyde or ketone groups either naturally occurring or synthetically produced on the biomolecule.

63. The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety is coupled to the trifunctional cross-linking moiety without the third linker.

64. The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety is coupled to the trifunctional cross-linking moiety with a third linker comprising a spacer having a length of 1-25 atoms, or groups of atoms.

65. The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety is coupled to the trifunctional cross-linking moiety with a third linker comprising a spacer having a length of 6-18 atoms, or groups of atoms.

66. The single molecule reagent according to claim 33, wherein the third linker comprises at least one hydrogen- bonding atom.

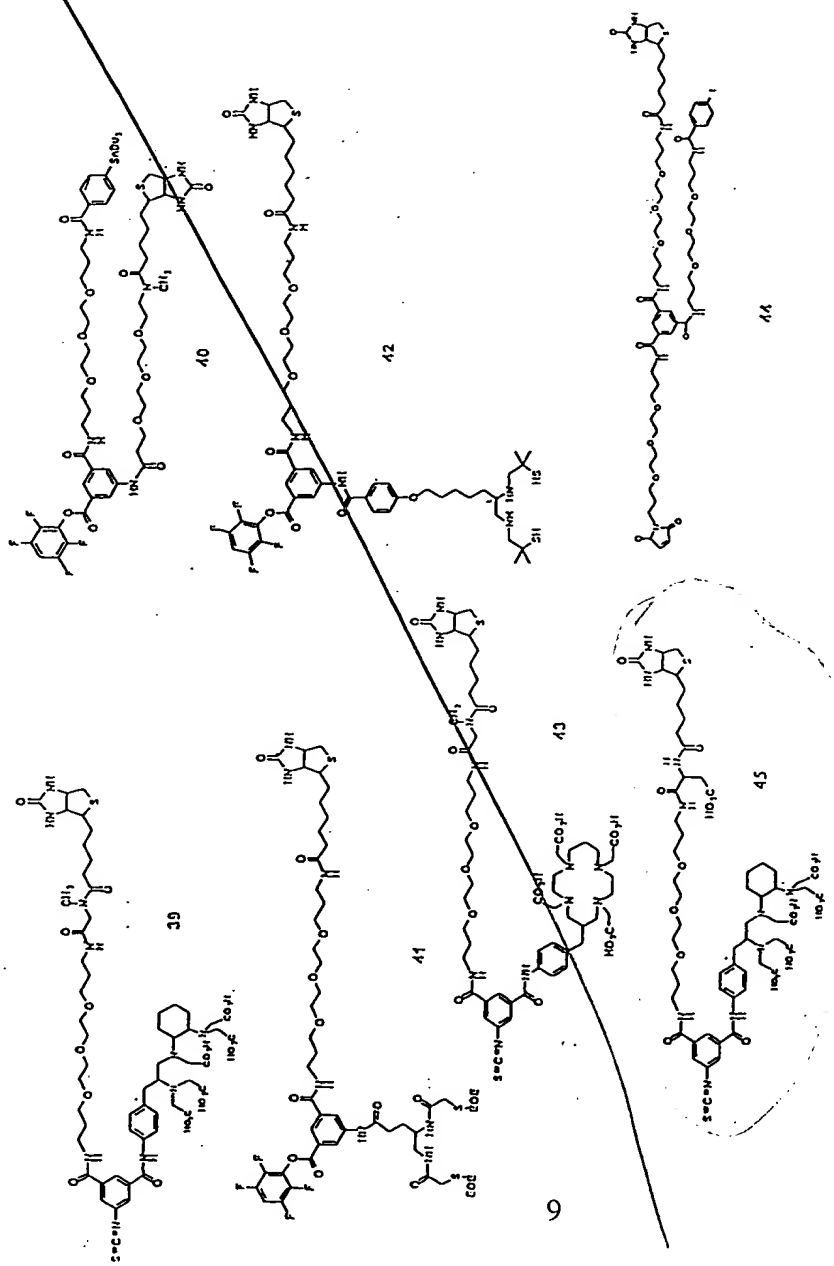
67. The single molecule reagent according to claim 66, wherein the hydrogen-bonding atom comprises a member selected from the group consisting of ethers and thioethers.

68. The single molecule reagent according to claim 33, wherein the third linker comprises an

ionizable group.

69. The single molecule reagent according to claim 68, wherein the ionizable group comprises a member selected from the group consisting of carboxylates, sulfonates and ammonium groups.

70. The single molecule reagent according to claim 33, wherein the reagent is a member selected from the group consisting of the following compounds:



71. The single molecule reagent according to claim 33, wherein more than one affinity ligand is bound to the trifunctional cross-linking moiety, which is tetrafunctional.

72. The single molecule reagent according to claim 33, wherein more than one effector agent is bound to the trifunctional cross-linking moiety, which is tetrafunctional.

73. A reagent for the diagnosis of a condition or disease in a mammal, the condition or disease selected from the group consisting of cancer, myocardial infarction, deep vein thrombosis, stroke loci, pulmonary embolism and atherosclerosis, the reagent comprising the single molecule reagent according to claim 33.

74. A reagent for the treatment of a condition or disease in a mammal, the condition or disease selected from the group consisting of cancer, myocardial infarction, deep vein thrombosis, stroke loci, pulmonary embolism and atherosclerosis, the reagent comprising the single molecule reagent according to claim 33.

75. A method of detecting an affinity label bound to a biomolecule, comprising:

labeling the biomolecule with biotin or a derivative thereof having a similar affinity by conjugation of the single molecule reagent according to claim 33, the reagent comprising an affinity ligand having affinity for biotin or a derivative thereof and a detectable effector agent; and

detecting the amount of affinity ligands of the reagent conjugated to the biomolecule.

76. A method for diagnosing a condition or disease in a mammal, comprising:

conjugating a biomolecule to the reagent according to claim 33 to obtain a conjugated biomolecule;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such diagnosis, such that the conjugated biomolecule is concentrated at a target site at which the reagent is to be detected;

optionally removing from the blood circulation of the mammal any amount of conjugated biomolecule not concentrated at the target site to be detected, by at least one method selected from the group consisting of:

administering to the mammal a protein which specifically binds to the affinity ligand, and

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand; and

detecting the intended target site specific for the conjugated biomolecule.

77. A method for treating a condition or disease in a mammal, comprising:

conjugating a biomolecule to the reagent according to claim 33 to obtain a conjugated biomolecule;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such treatment, such that the conjugated biomolecule is concentrated at a site at which the conjugated biomolecule is to exert a therapeutic action; and

optionally removing from the blood circulation of the mammal any amount of

such conjugated biomolecule not concentrated at the site at which the reagent is to exert its therapeutic action, by at least one method selected from:

administering to the mammal a protein which specifically binds to the affinity ligand, or

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand.

78. A method for diagnosing a condition or disease in a mammal, comprising:

conjugating a biomolecule to the reagent according to claim 33 to obtain a conjugated biomolecule, wherein the reagent is provided with a radionuclide either before or after conjugation of the biomolecule to the reagent;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such diagnosis, such that the conjugated biomolecule is concentrated at a target site at which the reagent is to be detected;

optionally removing from the blood circulation of the mammal any amount of conjugated biomolecule not concentrated at the target site to be detected, by at least one method selected from the group consisting of:

administering to the mammal a protein which specifically binds to the affinity ligand, and

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand; and

detecting the intended target site specific for the conjugated biomolecule.

79. A method for treating a condition or disease in a mammal, comprising:

conjugating a biomolecule and a radionuclide to the reagent according to claim 33 to obtain a conjugated biomolecule, wherein the reagent is provided with a radionuclide either before or after conjugation of the biomolecule to the reagent;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such treatment, such that the conjugated biomolecule is concentrated at a site at which the conjugated biomolecule is to exert a therapeutic action; and

optionally removing from the blood circulation of the mammal any amount of such conjugated biomolecule not concentrated at the site at which the reagent is to exert its therapeutic action, by at least one method selected from:

administering to the mammal a protein which specifically binds to the affinity ligand, or

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand.

80. A method of detecting an affinity label bound to a biomolecule, comprising:

labeling a biomolecule with an affinity label by conjugation of the reagent according to claim 33 to obtain an affinity-labeled biomolecule; and

determining an activity and thereby an amount of the effector agent of the reagent,

wherein an amount and activity of the effector agent is proportional to the number of affinity ligands on the biomolecule.

81. A kit for diagnosing a condition or disease in a vertebrate host, comprising:

a diagnostic biomolecule;

the single molecule reagent according to claim 33;

an optional plasma separation device for separation of plasma from blood;

optional means for extracorporeal circulation of whole blood or plasma from the vertebrate host; and

an optional extracoporeal adsorption device comprising immobilized receptors specific toward the affinity ligand of the single molecule reagent.

B1

82. A kit for treating a condition or disease in a vertebrate host, comprising:

a therapeutic biomolecule;

the single molecule reagent according to claim 33;

an optional plasma separation device for separation of plasma from blood;

optional means for extracorporeal circulation of whole blood or plasma from the vertebrate host; and

an optional extracoporeal adsorption device comprising immobilized receptors specific towards the affinity ligand of the single molecule reagent.

83. The kit according to claim 81, wherein the effector agent of the single molecule reagent is selected from the group consisting of radionuclide binding/bonding moieties with or without the

radionuclide.

84. The kit according to claim 82, wherein the effector agent of the single molecule reagent is selected from the group consisting of synthetic or naturally occurring toxins, enzymes capable of converting a pro-drug to an active drug, immunosuppressive agents, immunostimulating agents, and radionuclide binding/bonding moieties with or without the radionuclide.

85. The kit according to claim 81, wherein the affinity ligand comprises biotin, or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin, and wherein the immobilized receptor comprises a member selected from the group consisting of avidin; a derivative, mutant or fragment of avidin having essentially the same binding function to the affinity ligand as avidin; streptavidin; and a derivative, mutant or fragment of streptavidin having essentially the same binding function to the affinity ligand as streptavidin.

86. The kit according to claim 82, wherein the affinity ligand comprises biotin, or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin, and wherein the immobilized receptor comprises a member selected from the group consisting of avidin; a derivative, mutant or fragment of avidin having essentially the same binding function to the affinity ligand as avidin; streptavidin; and a derivative, mutant or fragment of streptavidin having essentially the same binding function to the affinity ligand as streptavidin.

87. A method of using the kit according to claim 81, comprising:

conjugating the single molecule reagent with the diagnostic biomolecule to

obtain a conjugated biomolecule;

adding the conjugated biomolecule to the blood circulation of the vertebrate host, such that the conjugated biomolecule is concentrated at a target site to be detected;

optionally extracorporeally circulating the whole blood or plasma from the vertebrate host by means of the means for extracorporeal circulation;

optionally separating the plasma from the blood by means of the plasma separation device; and

optionally adsorbing any conjugated biomolecule in the blood or plasma by means of the optional extracoporeal adsorption device.

88. A diagnostic or therapeutic conjugate which can be extracorporeally eliminated from the blood of a mammal to which it is administered to minimize undesired toxic side-effects, the conjugate comprising:

an optionally modified biomolecule, having a desired biological property retained; and

the single molecule reagent according to claim 33, having at least one affinity ligand and at least one effector agent bound to the reagent.

89. A method of making a diagnostic or therapeutic conjugate which can be extracorporeally eliminated from the blood of a mammal to which it is administered to minimize undesired toxic side-effects, the method comprising reacting:

an optionally modified biomolecule, having a desired biological property retained; and

the single molecule reagent according to claim 33, having at least one affinity ligand bound and at least one effector agent bound to the reagent.

90. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;

the affinity ligand comprises biotin;

the second linker comprises an aminobenzyl group; and

the biomolecule reactive moiety comprises an isothiocyanate.

B1

91. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;

the affinity ligand comprises biotin;

the second linker comprises a trioxadiamine; and

the biomolecule reactive moiety comprises a tetrafluorophenyl ester.

92. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;

the affinity ligand comprises homobiotin;

the first linker comprises a trioxadiamine;

the second linker comprises a propionate moiety; and

the biomolecule reactive moiety comprises a tetrafluorophenyl ester.

93. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;

the affinity ligand comprises homobiotin;

the first linker comprises a trioxadiamine;

the second linker comprises a pentyloxybenzoate group; and

the biomolecule reactive moiety comprises a tetrafluorophenyl ester.

94. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;

the affinity ligand comprises biotin;

the first linker comprises a biotinidase-stabilizing linker;

B1 the second linker comprises an amibenzyl group; and

the biomolecule reactive moiety comprises an isothiocyanate.

95. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises tricarboxybenzene;

the affinity ligand comprises biotin;

the first linker comprises a biotinidase-stabilizing linker;

the second linker comprises a trioxadiamine moiety;

the third linker comprises a trioxadiamine moiety; and

the biomolecule reactive moiety comprises a maleimide group.

96. The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;

the affinity ligand comprises biotin;

the first linker comprises a biotinidase-stabilizing linker;

the second linker comprises an aminobenzyl group; and

the biomolecule reactive moiety comprises an isothiocyanate.

97. A method for the detection of affinity ligand bound to a biomolecule, comprising

labeling the biomolecule with biotin or a derivative thereof having a similar affinity, by conjugation of the single molecule reagent according to claim 33, the reagent comprising an affinity ligand having affinity for biotin or a derivative thereof; and

detecting an amount of affinity ligand of the reagent conjugated to the biomolecule.

If any additional fees are due in connection with this filing, such as additional fees under 37 C.F.R. §§ 1.16 or 1.17, please charge the fees to our Deposit Account No. 02-4300. If an extension of time under 37 C.F.R. § 1.136 is necessary that is not accounted for in the papers filed herewith, such an extension is requested. The additional extension fee also should be charged to Deposit Account No. 02-4300. Any overpayment can be credited to Deposit Account No. 02-4300; Order No. 033700.005.

Respectfully submitted,

SMITH, GAMBRELL & RUSSELL, LLP

By:  Reg No. 42,391
for Robert G. Weilacher, Reg. No. 20,531
1850 M Street, N.W., Suite 800
Washington, D.C. 20036
Telephone: (202) 659-2811
Fax: (202) 263-4329

June 22, 2001

RGW/MKC:jvh